

MICRODETERMINATIONS OF SOME SULFUR COMPOUNDS BY MEANS OF THE IODINE-AZIDE REACTION

by

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1. INTRODUCTION

As stated in the preceding paper (LØVTRUP (9)) the original aim of the present investigation was to study the iodine-azide reaction as an analytical tool for the compounds. It was hoped that the utilization of catalytical action, combined with the possibility of measuring small gas volumes in the Cartesian diver (8), would permit the estimation of rather small quantities of sulfides. This hope was not unjustified; we have found that cystine for instance can be determined in amounts of $1 \times 10^{-3} \mu\text{g}$ with an accuracy of a few percent. On the other hand it turned out that the analytical utility of the reaction is somewhat limited by the fact that not all sulfides act as true catalysts in the sense that they emerge completely unchanged at the end of the reaction. Some of them, e. g. the *SH*-compounds are oxidized by iodine at such a rate as to make their determination impossible (FRIEDMAN (3)). Others, like the *SS*-compound cystine are also oxidized but, as shown by LØVTRUP (9), at a rate which still permits the determination of the initial concentration. Finally there is a group of substances, like carbondisulfide and the polythionates (both studied by HOFMAN-BANG (4)) which are not used up in side-reactions and thus behave essentially as true catalysts.

In the following report we shall describe the determination of one representative of each of the two last groups mentioned, namely, tetrathionate and cystine. Since the kinetics of the reactions involved is different, the diver technique had to undergo some slight adjustments in order to suit the special requirements of the two cases.

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2. EXPERIMENTAL

2.1. Tetrathionate.

In these experiments divers of the standard type (HOLTER (5) fig. 7a) were employed. Into the bottom of the diver was pipetted $1.0\ \mu\text{l}$ of a solution of the following composition: $0.05\ M$ sodium azide, $0.025\ M$ iodine and $0.05\ M$ potassium iodide. To this was added $0.5\ \mu\text{l}$ (expt. 1, table 1) or $0.1\ \mu\text{l}$ (expts. 2 and 3, table 1) containing the amount of tetrathionate listed in the table. The solutions were pipetted by means of calibrated pipettes of type I (HOLTER (5), fig. 13). The oil seal and the mouth seal were placed as quickly as possible after addition of the catalyst, the diver was placed in the thermostat at 22° , and 10-15 minutes after the addition of the catalyst the first reading was taken.

It will be noticed that in the arrangement here described the reagents are mixed before the diver is closed. This is only permissible if the rate of catalysis is linear long enough to be measured with sufficient accuracy. This is the case as long as the concentration of azide is high enough to be considered constant. As fig. 1 shows, this means in practice that under the conditions of our experiments a total N -evolution of $0.2\ \mu\text{l}$ falls within the range of linearity.

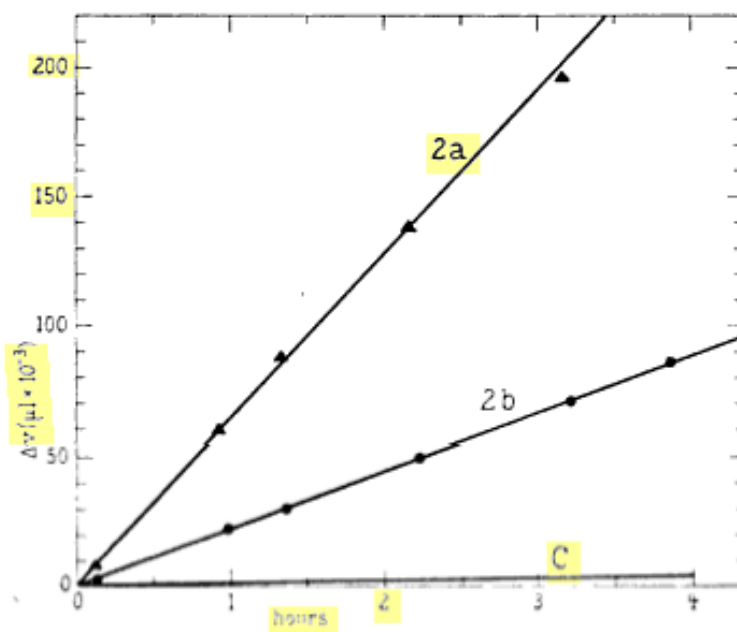


Fig. 1. Example of tetrathionate catalysis. 2a and 2b are the same experiments as listed in table 1. C is the blank.

Fig. 1 gives an example of the results obtained. The rate of reaction is determined as the slope of the (graphically smoothed) straight line obtained when the nitrogen evolution is plotted against time. From the values of the slopes experimentally found is subtracted $1.2 \mu\text{l} \times 10^{-3}/\text{hr}$ which is the average of many blank divers, charged with iodine and azide, but without catalyst. It will be noticed that these blanks register a slight gas evolution, contrary to the ordinary control divers, which become slightly heavier with time. This is probably due either to a spontaneous iodine-azide reaction or to the presence of minute amounts of impurities.

Table I shows the results of three experiments done on different days. They all show good proportionality between amount of catalyst and nitrogen evolution, but they are not directly comparable to each other, for the following reasons: In the divers of expt. I the catalyst was added together with $0.5 \mu\text{l}$ of water, in experiments 2 and 3 with only $0.1 \mu\text{l}$, while the amount of iodine-azide solution

Table 1.
Tetrathionate catalysis.

date	experiment	$\Delta N_2/\Delta t$ ($\mu\text{l} \times 10^{-3}/\text{hr}$)	tetrathionate ($\mu\text{g} \times 10^{-3}$)	$\frac{\Delta N_2}{\Delta t \times [\text{tetrathionate}]}$ ($\mu\text{l}/\mu\text{g} \times \text{hr}$)
9-I	1 a	72.6	75	0.97
9-I	1 b	23.1	25	0.93
14-I	2 a	63.0	37.5	1.68
14-I	2 b	21.0	12.5	1.68
22-I	3 a	47.5	25	1.83
22-I	3 b	15.2	8.3	1.83

was the same ($1 \mu\text{l}$) in all cases. In other words, not only the concentration of catalyst but also that of azide was different in the two sets of experiments. Since the rate of nitrogen evolution depends both on catalyst and azide concentration, no agreement can be expected.

This fact can roughly be corrected for. Assuming from the results of HOFMAN-BANG (4) that the rate of nitrogen evolution is proportional to the azide concentration as well as to the catalyst it follows that if we multiply 1.68 with the square of the ratio of volumes $\left(\frac{1.1}{1.5}\right)^2$ we will arrive at the rate at which experiment 2 would proceed, if the same absolute amounts of azide and catalyst were present in $1.5 \mu\text{l}$ instead of $1.1 \mu\text{l}$. This correction gives 0.91, in sufficient agreement with 0.95, the average actually found.

Experiments 2 and 3 were done with the same reagents and the same amounts of solutions but they, too, are not quite comparable because the solution of catalyst was fresh in expt. 2 and 8 days old in expt. 3. Now it is known from the work of KURTENACKER, MUTSCHIN and STASTNY (6) that solutions of tetrathionate on standing are slowly converted to a mixture of tri- and pentathionate. According to HOFMAN-BANG (4), the catalytic activity of trithionate is of the same order as that of tetrathionate, whereas the corresponding velocity constant for pentathionate is many times higher. Thus we would expect the catalytic activity of a tetrathionate solution to rise on standing, in agreement with our results.

The sensitivity of the reaction is best indicated by the nitrogen "turnover" number, which in experiment 2 (fresh tetrathionate solution) amounts to 20.4 molecules N_2 evolved per molecule tetrathionate and per hour.

2.2. *Cystine.*

In a preceding paper (LØVTRUP 9)) it was found that if the iodine-azide reaction is catalyzed by cystine, the rate of N_2 -evolution changes with time because of the cystine oxidation proceeding simultaneously. Thus it was impossible in this case to determine the catalytic activity by measuring constant rates of reaction, and we were forced to measure total N_2 -evolution, which was found to be proportional to the cystine concentration at zero time.

Under these circumstances the method employed in the tetrathionate experiments had to be changed. The main requirement was now to have the diver equilibrated before the catalyst was added to the azide-iodine solution. The problem of mixing two drops inside the diver had arisen previously and was solved by coating the inside of the diver neck with paraffin (ANFENSEN and CLAFF (1) or with silicone (SCHWARTZ (10)). In both cases one of the solutions to be mixed is placed as a drop on the side of the diver wall, while the other is placed as a neck seal which can be moved and mixed with the side drop by the application of a suitable overpressure. The hydrophobic coating prevents premature coalescence of the two solutions.

Of the two methods we chose the one by SCHWARTZ (10) as the silicone coating is easier to apply and more convenient to work with. The experiments were performed in cylindrical divers (HOLTER (5) fig. 7c). This decreased the gas volume of the divers about

50 percent, which meant a corresponding increase of the sensitivity of the method. The cystine solution was placed on the side of the diver neck by means of the pipette shown in fig. 2, which is a combination of a constriction pipette (LEVY (7)) and a braking pipette (HOLTER (5)).



Fig. 2.
0.17 μ l
constriction
pipette.

It consists of a piece of thinwalled glass-tubing, drawn out to capillaries in both ends and mounted in a glass jacket by means of picein. The tip of the pipette is bent to form a little hook, which facilitates placing of the side drop. The capillary at the other end (the brake) contains two constrictions. These are easily made by means of a platinum wire loop, heated to glowing by an electrical current. The process of making the constriction must be watched through a strong lens or a dissection microscope. Since we had a FONBRUNE micro-forge at our disposal (2), we have used this for making these fine constrictions. The pipette used in the experiments described here had a volume of 0.168 μ l between the constrictions. The volume was determined iodometrically, by pipetting off 1 N iodate solution into potassium iodide. The results of five deliveries were 0.168, 0.169, 0.172, 0.163, and 0.168; average: 0.168 μ l, standard deviation: 0.003 μ l. It is to be assumed that part of the standard deviation is due to titration errors, and that the accuracy of delivery is even better.

0.4–0.5 mm above the side drop a seal containing azide, iodine and buffer was placed. Volume of this seal: 0.6 μ l, concentrations of reagents: 0.29 M NaN_3 , 0.03 M I_2 in 0.07 M KI, and 0.07 M citratebuffer pH 4.5. A 0.5 μ l oil seal and a mouth seal were placed in the usual manner, and the diver was placed in the 22° thermostat. During the two first hours some readings were taken to measure the “blank” evolution of N_2 , then the cystine was mixed with the iodineazide drop.

During the course of the reaction, some iodine is lost from the azide-iodine seal by distillation into the oil seal. In the case of tetrathionate this does not matter, since the rate of the reaction is independent of the iodine concentration (HOFMAN-BANG (4)). In the case of cystine too, the phenomenon can be neglected because, although the rate of nitrogen evolution does depend on the iodine concentration (LØVTRUP (9)), the total amount of nitrogen liberated does not.

The reaction proceeded in complete agreement with the results obtained in the WARBURG apparatus (LØVTRUP (9)), following the course of a first order reaction (fig. 3.), if the rate of the “blank” N_2 -evolution is subtracted from the total rate of reaction.

It is of course unnecessary to read the pressure during the

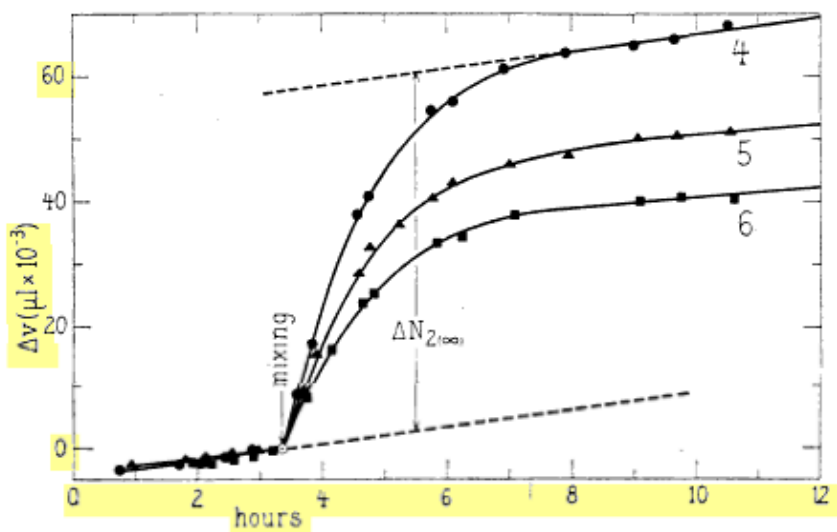


Fig. 3. Example of cystine catalysis. 4, 5, and 6 are the same experiments as listed in table 2. In case 4 is indicated the way of calculating $\Delta N_{2(\infty)}$.

reaction since we are interested only in total N_2 -evolution. In the experiments represented in fig. 3, it would have been sufficient to measure the N_2 -evolution before the reaction started, then mix and wait till the reaction was stopped (5 hrs) and then take a few readings again. The interval of 5 hours might be reduced considerably by increasing the iodine concentration, which was rather low in the experiments here reported.

The amount of nitrogen evolved is obtained by measuring the vertical distance between the two parallel lines (broken lines on fig. 3) which indicate the rate of nitrogen evolution before mixing

Table 2.
Cystine catalysis.

date	experiment	$\Delta N_{2(\infty)}$ ($\mu\text{l} \times 10^{-3}$)	cystine ($\mu\text{g} \times 10^{-3}$)	$N_{2(\infty)}/\text{cystine}$ ($\mu\text{l}/\mu\text{g}$)
13-II.....	1	61.1	1.69	36.2
13-II.....	2	45.1	1.27	35.5
13-II.....	3	31.2	0.85	36.7
16-II.....	4	59.5	1.69	35.2
16-II.....	5	44.5	1.22	36.5
16-II.....	6	34.5	0.92	37.4

and after completion of cystine catalysis. The pressure difference thus obtained is then converted in the usual way into $\Delta v = \Delta N_2(\infty)$, the nitrogen evolution in $\mu\text{l} \times 10^{-3}$. Table 2 shows the results of 2 experiments.

The run of Nov. 16 is the one represented in fig. 3. According to these data, the nitrogen evolution is 185 molecules per molecule cystine.

CONCLUSION AND SUMMARY.

The catalysis of the iodine-azide reaction by sulfides can be utilized for the quantitative determination of certain sulfur compounds. If performed in the Cartesian diver, the reaction can be developed into a very sensitive micro determination. Tetrathionate was studied as a representative of a true catalytic substance, causing a gas evolution which proceeds at a practically constant rate for several hours. In this case, amounts of $10 \times 10^{-3} \mu\text{g}$ tetrathionate could be determined with an accuracy of 2-3%.

In the case of substances like cystine which are subject to relatively slow side reactions, quantitative determination is still possible if total gas evolution is measured instead of reaction rates. Thus $1 \times 10^{-3} \mu\text{g}$ cystine could be determined with an accuracy of 2.5% (standard deviation).

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REFERENCES

1. ANFINSEN, C. B. and C. LLOYD CLAFF: An extension of the Cartesian diver micro respirometer technique. *J. Biol. Chem.* **167**, 27-33 (1947).
2. FONBRUNE, P. DE: *Micromanipulateur pneumatique et microforge pour la fabrication des micro-instruments*. Paris. 1937.
3. FRIEDMAN, E.: Sulfhydrylverbindungen als Katalysatoren der Natriumazidzersetzung durch Jod. *J. prakt. Chemie, N. F.* **146**, 179-192 (1936).
4. HOFMAN-BANG, N.: Personal communication.
5. HOLTER, H.: Technique of the Cartesian diver. *Compt. rend. Lab. Carlsberg, Sér. chim.* **24**, 399-478 (1943).
6. KURTENACKER, A., A. MUTSCHIN und F. STASTNY: Selbstzersetzung von Polythionatlösungen. *Z. anorg. und allg. Chemie.* **224**, 399-419 (1935).
7. LEVY, M.: Studies on enzymatic histochemistry XVII. A micro Kjeldahl estimation. *Compt. rend. Lab. Carlsberg, Sér. chim.* **21**, 101-110 (1936).
8. LINDERSTRØM-LANG, K.: Principle of the Cartesian diver applied to gasometric technique. *Nature*, **140**, 108. (1937).
9. LØVTRUP, S.: Observations on the kinetics of the cystine catalysis of the iodine-azide reaction. *Compt. rend. Lab. Carlsberg, Sér. chim.* **27**, 63-71 (1949).
10. SCHWARTZ, S.: The use of silicone coating on the Cartesian diver microgasometer. *Compt. rend. Lab. Carlsberg, Sér. chim.* **27**, 79-92 (1949).